we have developed techniques that use deuterium (^{2}H) solid state NMR to study AMP-membrane interactions in the context of intact bacteria. In particular we are investigating the correlation between AMP-induced lipid chain disorder in intact bacteria and biological function, as measured by minimal inhibitory concentration assays.

1216-Pos Board B108

Molecular Dynamics Simulations of Cod Antimicrobial Peptide Paralogs in Self-Assembled Bilayers

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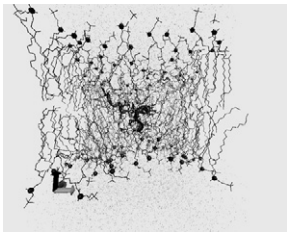
Gaduscidin-1 and −2 (GAD-1 and GAD-2) are antimicrobial peptides (AMPs) that are histidine-rich and thus are expected to exhibit pH dependent activity. In order to help explain their mechanism of membrane disruption, we have performed molecular dynamics simulations with the peptides in both histidine-charged and histidine-neutral forms, along with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipid molecules. The simulations employed GROMACS software and an OPLS-AA force-field. Initially, the peptide and lipids were placed randomly in the simulation box and then were allowed to self-assemble. The peptides were found to associate with defects in the bilayer, and to take on a variety of structures and topologies. The observed heterogeneity is consistent with experimental studies with similar peptides, and has potential to help explain the mechanism by which the peptides can incorporate into and disorder lipid bilayers.



1217-Pos Board B109

Modulation of Elastic Properties through Unsaturated Lipid Content as a Mechanism for Inducing Resistance to Amphiphilic Antimicrobial Peptides

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We explore whether changes in fatty acid composition in bacterial membrane models lead to inhibition of amphiphilic antimicrobial peptide-(AMPs) through the modulation of the membrane elastic properties. Specifically, we examined whether changes in the unsaturated/saturated fatty acid ratio, which is controlled by bacteria, inhibit AMP function. In phosphatidylglycerol (PG) lipid bilayers we observe a 10-fold increase in resistance to Magainin-2 (MAG) in POPG (16:0/18:1) vs. DPPG (16:0/16:0) bilayers and a 20-fold increase in resistance in DOPG (18:1/18:1) vs. DPPG bilayers, as measured by calcein release at 45 C (where lipids are in the liquid-crystalline phase). By analyzing the leakage kinetics, we find that the activation energy for pore formation in DPPG/POPG mixtures increases linearly with POPG content, confirming that unsaturation is energetically unfavorable for pore formation. Laurdan polarization (GP) measurements correlate well with MAG resistance, suggesting that head group spacing is an indicator of resistance (increased head-group spacing could allow for more peptides to partition into the head group region, instead of becoming inserted into the bilayer as bilayer-spanning monomers). The changes in potency cannot be explained solely by the increased head group spacing, however. We therefore performed leakage experiments using known modifiers of membrane elastic properties (Triton-X, capsaicin) that both reduce bilayer stiffness but cause opposite changes in curvature. Bilayer softening plays a role in decreasing MAG's potency, whereas the curvature alters only the leakage kinetics, not potency. Based on previous studies, we propose that membrane softening somehow makes it more difficult for MAG to reach a critical concentration necessary to produce pore formationmaybe because the energetics of lateral association among bilayer-spanning MAG monomers favors the monomers in softer bilayers. Unsaturated lipids may therefore influence MAG potency by modulating membrane stiffness.

1218-Pos Board B110

Peptide-Induced Bilayer Thinning Structure of Unilamellar Vesicles and the Related Binding Behavior as Revealed by X-ray Scattering

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To quantitatively correlate membrane thinning with peptide binding affinity, we have studied the bilayer thinning structure of unilamellar vesicles (ULV) of a phospholipid 1,2-dierucoyl-*sn*-glycero-3-phosphocholine (di22:1PC) upon binding of melittin, a water-soluble amphipathic peptide. Successive thinning of the ULV bilayers with increasing peptide concentration was monitored via small-angle X-ray scattering (SAXS). Results suggest that the two leaflets of the ULV of closed bilayers are perturbed and thinned asymmetrically upon free peptide binding, in contrast to the centro-symmetric bialyer thinning of the substrate-oriented multilamellar membranes (MLM) with premixed melittin. Moreover, thinning of the melittin-ULV bilayer saturates at ~4 %, significantly lower than the critical thinning of ~8% (determined via the correspondingly premixed peptide-MLM bilayers) for thermally equilibriated formation of membrane pores, revealing a critical influence of binding affinity for water soluble peptides. Scaling the peptide-ULV bilayer thinning to that of the corresponding peptide-MLM, of a calibrated peptide-to-lipid ratio, we have deduced the number of bound peptides on the ULV bilayers as a function of free peptide concentration in solution. The hence derived X-ray-based binding isotherm allows extraction of a low binding constant for melittin to the ULV bilayers, on the basis of surface partition equilibrium and the Gouy-Chapman theory. Moreover, we show that the ULV and MLM bilayers of di22:1PC may have a same thinning constant upon binding of a same peptide, hence providing a basis in establishing X-ray-based binding isotherms for thermodynamic binding parameters of late stage peptide-membrane interactions prior to pore formation.

1219-Pos Board B111

Molecular Basis of the Blocking Mechanism of Inwardly Rectifying Channels by Tertiapin

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Ion channels are the third largest type of proteins targeted by pharmaceutical industry accounting for 10% of the currently marketed drugs. Recent advances in the 3-D structural determination of ion channel proteins coupled with highly sensitive electrophysiological assays make a compelling case for rational drug design targeting these proteins. High-resolution structure determination of inwardly rectifying Potassium (Kir) channels has thus far yielded two full-length mammalian structures. Specific blockers for Kir channels do not exist but the small bee venom peptide toxin tertiapin (TPN) targets with high affinity two Kir channels: ROMK1 and GIRK channels. Mutations of ROMK1 in the kidney cause hypotension, while, malfunctions of GIRK in the heart are associated with chronic atrial fibrillation. Modification of TPN to specifically target one or the other channel bears great therapeutic potential. We have produced structural models of TPN binding to ROMK1 and GIRK2 channels and have quantified the energies involved for each channel-toxin interaction pair. *In silico* mutagenesis has produced remarkably similar changes to the experimental energies of interaction between the ROMK1 channel and TPN, lending strong validation to the structural model employed. Similar studies in GIRK2-TPN interactions suggest differences in specific interactions of the toxin with each of the two channels. These models make specific predictions that are being pursued for further experimental validation. The underlying hypothesis of our approach is that differences in the interaction energies we compute can guide the design of specific TPN for different Kir channels.

1220-Pos Board B112

Membrane Permeability Induced by Stereo and Retro Analogs of Histatin 5

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Candida species combine to be the major cause of fungal infections in humans. These infections are becoming increasingly difficult to treat due to resistance to current common drug therapies or due to the toxicity of other therapeutic compounds. Antimicrobial peptides, short peptides of less than 100 amino acid residues, are known to kill bacterial and fungal pathogens, with the potential to be a more reliable means of treatment for *Candida* infections than the drugs currently available. Humans naturally produce the 24 amino acid cationic histatin 5 peptide. Variants of a 16 amino acid peptide derived from histatin 5 were chemically synthesized to delineate the structural determinants of their antifungal activity. *In-vitro* acridine orange leakage assays were used to evaluate the activity of histatin 5 derivatives with various synthetic membrane bilayers (liposomes) composed of differing lipid and sterol compositions. The variants of histatin 5 were synthesized with alternative stereochemistry and polarity, and then evaluated with liposomes comprised of either Soy PhosphatidylCholine (Soy PC), Soy PC + cholesterol or Soy PC + ergosterol. The results indicate that neither the D or L amino acid stereochemistry nor the N- to C- terminal

polarity of the amino acid chain is influential in the induced membrane permeability of these peptides. In addition, these *in-vitro* studies imply the existence of a factor or a combination of factors aside from strictly membrane sterol content that determines the specificity of these antimicrobial peptides for fungal cells *in-vivo*. Importantly, the results of this study imply that the relative spatial position of the amino acids in histatin 5 is critical for the antifungal activity.

1221-Pos Board B113

Visualizing pHLIP Insertion in Plasmamembrane and Endosomal Membrane

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The pH-(Low) Insertion Peptide (pHLIP) has potential as a tumor-targeting drug carrier. At neutral pH, pHLIP has affinity for the surface of a lipid bilayer, whereas under slightly acidic conditions (e.g. pH ~ 6) pHLIP inserts into the membrane, forming a transmembrane helix. Since many solid tumors are more acidic than healthy tissues, pHLIP may be used to translocate chemotherapeutic agents selectively into cancer cells. Knowledge of the exact location of pHLIP insertion in cells can guide the rational design of delivery constructs. We envision two scenarios for pHLIP insertion in cells: First, pHLIP may directly insert into the plasmamembrane; alternatively, cells may internalize pHLIP molecules via endocytosis, and subsequently pHLIP may insert into the endosomal membrane. In this study, several fluorescently self-quenched pHLIP constructs were synthesized to visualize to what extend these two scenarios are occurring in cells at pH 7.3 and 6.2. In these self-quenched pHLIP constructs, a rhodamine dye (TAMRA or Alexa Fluor 568) is attached to a C-terminal Lys residue, with the quencher QSY-9 conjugated to an adjacent Cys via a cleavable disulfide linker (or a stable thio-ether bond). Upon insertion, pHLIP would translocate its C-terminus into the cell cytoplasm, where cleavage of the disulfide linkage and release of the quencher QSY9 can take place. In turn, the pHLIP construct would become more fluorescent.

1222-Pos Board B114

Phospholipase A2 Activity and Substrate Specificity of Bothrops Asper and Crotalus Durissus Cumanensis Snake Venom Collected from the Guajira Region of Colombia

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Phospholipase A2 (PLA2) is a versatile enzyme present in all organisms that hydrolyzes the sn-2 ester bond of phospholipids resulting in the formation of lysophospholipids and free fatty acids. PLA2 found in snake venom is responsible for multiple systemic effects such as neurotoxicity, myotoxicity, and hemolysis. We measure the specificities of PLA2 in whole venom samples towards different phospholipids. This study provides a first look at the biophysical characteristics of venoms isolated from Colombia. Crude venom samples were taken from Bothrops asper and Crotalus durissus cumanensis in the Guajira region of Colombia. The venom was lyophilized until used. Vesicles composed of the non-hydrolyzable 1,2-di-O-octadecyl-sn-glycero-3-phosphocholine (DEthPC) carrying calcein at a self-quenching concentration (50mM) were used as reporter vesicles, and target vesicles (substrate) made from DMPG, DMPC, POPE or sphingomyelin (SM) were used for measuring snake venom specificity. All measurements were performed in a pc1 Fluorometer (ISS, Urbana, IL) between 20°C and 60°C with and without calcium (30 μM). In the presence of calcium both venoms have similar catalytic action with C. durissus being slightly higher than B. asper. In the absence of calcium B. asper showed the highest activity indicating differences in the intrinsic calcium levels in both venoms. Overall B. asper showed the lowest lagtime for all assays. No calcein release was observed for SM. DMPG was the only one that presented activity at all temperatures suggesting that both venoms have strong affinity towards negatively charged membranes. Both snake venoms showed activity in DMPC only in the presence of extrinsic calcium and only at 24°C, where liquid-crystalline/gel phase coexistence is present, suggesting that the presence of membrane defects plays an important role in the initiation of catalysis.

1223-Pos Board B115

Identification and Characterization of the Glycan Binding Site of Vibrio Cholerae Cytolysin

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Vibrio cholerae cytolysin (VCC) is a pore-forming toxin secreted by the Vibrio cholera O1 El Tor strain. VCC is hypothesized to perform an important function by lysing immune cells and thereby protecting the bacteria from host defenses. VCC consists of multiple domains including two possible carbohydrate-binding lectin domains (β-prism and β-trefoil). Recently it has been found that the β-prism domain binds to both mono and oligosaccharides, but interacts with highest affinity to glycans found on eukaryotic cell membranes. Crystal structures exist for VCC in water-soluble and membrane assembled forms, but the precise molecular mechanism by which VCC recognizes carbohydrates is still not well understood. Here we show that the Asp 617 residue in the β-prism domain plays an important role in both monosaccharide and cell surface glycan binding. Site directed mutagenesis of the Asp 617 to alanine resulted in a ~80-fold decrease in monosaccharide binding activity and a ~280-fold decrease in cell-surface glycan binding activity. Furthermore, the Asp 617 mutant displayed a decline in hemolytic activity compared to wild type VCC when incubated with rabbit red blood cells. We also show that both monosaccharides and purified cell surface glycans can compete with VCC and cause a dose dependent lag in the half-life of rabbit blood hemolysis. These results indicate that the VCC interacts with glycans on target cell membranes and Asp 617 is involved in this binding process. Having more insight into the molecular mechanism and residues involved in sugar recognition may enable the development of sugar-based therapies against VCC and other pore-forming toxins.

1224-Pos Board B116

Membrane Association of Diphtheria Toxin T-Domain Characterized by Coarse-Grained and Atomistic Molecular Dynamics Simulations and Experiments

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Acidification of the endosome interior induces a series of conformational changes in diphtheria toxin T-domain ultimately resulting in its membrane insertion and translocation of its N-terminus with the attached catalytic domain into the cytoplasm. Previously, we have used a combination of various computational and experimental approaches to characterize the first step of this complex pathway, namely the formation of the membrane-competent state. Here we extend this study to investigate how this refolded state interacts with surfaces of lipid bilayers in preparation for transmembrane insertion. We present the results of multiple microsecond coarse-grained molecular dynamics simulations of the T-domain performed in the presence of the lipid bilayers of various compositions. The MARTINI force-field was used in our simulations. Binding of the protein to the membrane was improved as the anionic content of the mixed POPG:POPC bilayer increases. We have identified two preferred surfaces on the T-domain structure that are likely to participate in the early stages of membrane binding. The first surface is comprised of residues in the loops of helices TH2-3, TH8-9 and helix TH5. The second preferred surface of contact contains residues of the N-terminal (helices TH1-4) and those located in the loop between TH7-8. The experimental results corroborate these observations. Detailed atomistic MD simulations are presented to estimate free energy of the protein-lipid association to further validate our models. Supported by NIH GM069783

1225-Pos Board B117

Membrane Interactions of the Alzheimer's Disease Aβ42 Peptide and a Soluble Aβ42 Fusion Protein

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The 42-residue amyloid-beta (Aβ42) peptide is a primary constituent of the neuritic plaques diagnostic of Alzheimer's disease, but its exact role in the disease remains unclear. Growing evidence supports that small, soluble oligomers of Aβ42 are responsible for neurodegeneration, perhaps through disruptive membrane interactions. However, the notorious propensity of the peptide to aggregate causes such interactions to be elusive to most studies. We have biosynthetically produced a highly soluble fusion construct, in high-yields (>100 mg/L of culture), from which native human Aβ42 can be cleaved and purified. Perturbations of phospholipid bilayers by the fusion construct are compared to Aβ42 peptide alone using differential scanning calorimetry and by 31P and 2H solid-state NMR measurements. Preliminary structural characterization is performed using circular dichroism and solution NMR on uniformly 15N, 13C-enriched protein.

1226-Pos Board B118

Structure- Function Relationship Studies of Piscidin 1 Bound to Model Tumor Cell Membranes

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